# Appendix A

# Acyclic polyacetals from polysaccharides

*Biomimetic biomedical "stealth" polymers*

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Technologically adaptable hydrophilic polymers combining negligible in vivo reactivity with biodegradability would be instrumental in the development of specialized materials for advanced biomedical applications. Such highly biocompatible biodegradable polymers can be obtained via partial emulation of carbohydrate interface structures prevalent in biological systems. These structures are also present in polysaccharides and in some cases can be chemically "carved out" and isolated as acyclic hydrophilic polyacetals.

## Introduction

Novel concepts in pharmacology and bioengineering impose new, more specific and more stringent requirements on biomedical polymers. Ideally, advanced macromolecular materials would combine negligible reactivity in vivo with low toxicity and biodegradability. Polymer structure should support an ample set of technologies for polymer derivatization; for example, conjugation with drugs, cell-specific ligands, or other desirable modifiers. Materials combining all the above features would be useful in the development of macromolecular drugs, drug delivery systems, implants and templates for tissue engineering.

On the chemistry level, developing such materials translates into an intricate problem of developing macromolecules with minimized interactions in vivo, completely biodegradable main chains, and readily and selectively modifiable functional groups. The problem is further aggravated by the fact that both the main chain and the functional groups interact with extremely complex biological milieu, and all their interactions may be amplified via cooperative mechanisms.

Macromolecule interactions *in vivo* are mediated by several components of cells surfaces, extracellular matrix, and biological fluids. For example, both macromolecule internalization by cells and cell adhesion to polymer-coated surfaces

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can be mediated by several cell surface elements, many of which are functionally specialized (phagocytosis- and endocytosis-associated receptors, adhesion molecules, etc). Macromolecule recognition by cell receptors is often mediated by specialized recognition proteins of plasma, such as immunoglobulins (i), fibronectins (ii,iii,iv), proteins of complement system (v,vi,vii,viii), soluble lectins (ix,x,xi,xii), vitronectin (xiii) etc. These proteins contain at least one receptor-recognizable site per molecule, and often more than one substrate-hinding site. Although other proteins, e.g., albumin, also can hind polymers (via non-specific mechanisms), the distinctive features of recognition proteins relate to their ahility to trigger remarkable hiological responses. Some recognition proteins, such as C-reactive protein, are acute-phase proteins, i.e., their concentration in plasma increases as a result of inflammation or trauma. Others, such as β2-glycoprotein I (β2GPI), are "reverse" acute phase proteins, i.e., their concentration in hlood during the acute phase decreases. Recognition proteins hind a variety of structures; we have reviewed their role in pharmacology of macromolecules and particulates in more detail elsewhere (xiv,xv).

Cooperative hinding, often referred to as "non-specific interactions", is another major factor of macromolecule (and surface) reactivity in vivo. Cell interactions with polymers and recognition protein-polymer complexes also have an element of cooperativity (xvi,xvii). The very nature of cooperative interactions suggests that *any* large molecule can significantly interact with a complex substrate, for the simple reason that, hecause the hinding energy is additive, the association constant of cooperative hinding ($K_a$) would grow with the numher of associations *exponentially* (xiv). In other words, any polymer of a sufficient length can be expected to interact with at least one of the various components of a hiological system. Even if a molecule of certain size shows low interactions in cell cultures and in vivo, a larger molecule of the same type, or a supramolecular assemhly, can have a much higher hinding activity (xviii).

The essence of the above is that even if polymer molecules are assemhled of domains that do not interact with cell receptors and recognition proteins, such molecules can he capahle of cooperative interactions in vivo, i.e., completely inert polymers may not exist at all. However, several hiomolecules and hiological interfaces do appear to he functionally inert, except their specialized signaling domains. For example, plasma proteins are known to circulate for several weeks without uptake in the reticuloendothelial system (RES), whereas artificial constructs of a similar size have never been reported to have comparahle hlood half-lives.

Hypothetically, the mutual "inertness" of the natural hiomolecules and surfaces may relate to their relatively uniform interface structures, where the potential hinding sites are always saturated hy naturally occurring counteragents present in ahundance. Therefore, emulation of the common interface structures can result in a material that would not actively interact with actually existing hinding sites hecause these sites would be pre-occupied by the natural "prototypes".

Poly- and oligosaccharides are the most ahundant interface molecules expressed (as various glycoconjugates) on cell surfaces, plasma proteins, and proteins of the extracellular matrix. Therefore, interface carbohydrates appear to be the best candidates for structural emulation. The main objective of the emulation is to identify

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and exclude all structural components that can be recognized, even with low affinity, by any biomolecule, especially by cell receptors and recognition proteins.

All interface carbohydrates have common structural domains, which appear to be irrelevant to their biological function. An acetal group and two adjacent carbons are present in all carbohydrates, whereas the receptor specificity of each molecule depends on the structure and configuration of the glycol domains of the carbohydrate rings (Figure 1). We hypothesized that biologically inert ("stealth") polymers could be obtained using substructures that form the acetal side of the carbohydrate ring, i.e., the -O-C-O- group and the adjacent carbons. Although functional groups that are common in naturally occurring glycoconjugates (e.g., OH groups) can be used as substituents, the potentially biorecognizable combinations of these groups, such as rigid structures at C1-C2-C3-C4 (in pyranoses), must be completely excluded. Positioning of the acetal groups within the main chain would ensure polymer degradability via proton-catalyzed hydrolysis.

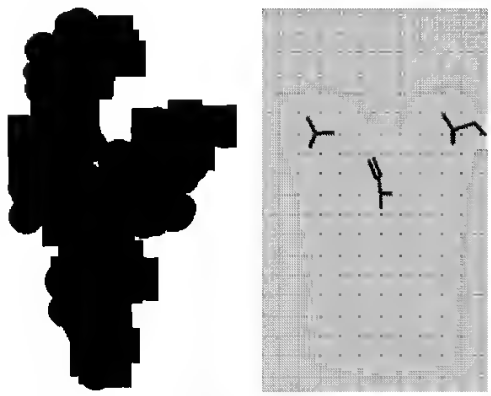

*Figure 1. The structure of oligosaccharide interface fragment of glycolipid $G_{M1}$ (space-filled and "stick" models of the same structure)*

*The signaling domains are shown in black; the biologically inert backbone in gray.*

Materials of the suggested general structure (acyclic hydrophilic polyacetals) can be produced using a variety of methods. For example, cleavage of potentially biorecognizable fragments from all carbohydrate residues of a polysaccharide would result in acyclic structures similar to that of interface carbohydrates. We used exhaustive periodate oxidation to transform (1->6)-poly-α-D-glucose into acyclic poly[carbonylethylene carbonylformal] (PCF) with subsequent borohydride reduction resulting in poly[hydroxymethylethylene hydroxymethylformal] (PHF). Both polymers, PCF and PHF, were isolated and characterized in order to evaluate the viability of the concept.

## Synthesis

Dextran B512, a product of *Leuconostoc Mesenteroides,* is a linear (1->6)-poly-α-D-glucose with ca. 5% (1->3; β) branching; 95% of the branches are only one or two

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residues long (xix). Periodate oxidation of 1->6 connected polysaccharides has been previously studied (xx). In unsubstituted pyranosides the periodate reaction, which is highly specific to 1,2-glycols, starts from breaking either C2-C3 or C3-C4 bond with formation of dialdehydes IIa or IIb. In dextrans, the kinetically controlled IIa/IIb ratio is approximately 7.5:1 (xx). The subsequent, slower stage results in the cleavage of carbon C3, with formation of dialdehyde III (Figure 2).

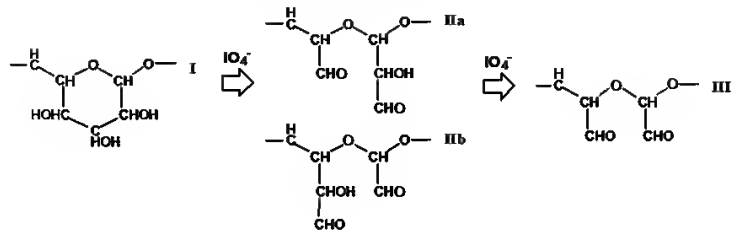


*Figure 2. Exhaustive periodate oxidation of an unsubstituted pyranose ring.*

Thus, exhaustive oxidation of an entirely 1->6 connected polysaccharide is expected to occur without depolymerization, resulting in macromolecular poly-[carbonylethylene carbonylformal] (PCF). The aldehyde groups can be subsequently reduced with borohydride to obtain a hydroxymethyl-substituted polymer, poly-[hydroxymethylethylene hydroxymethylformal] (PHF, Figure 3).

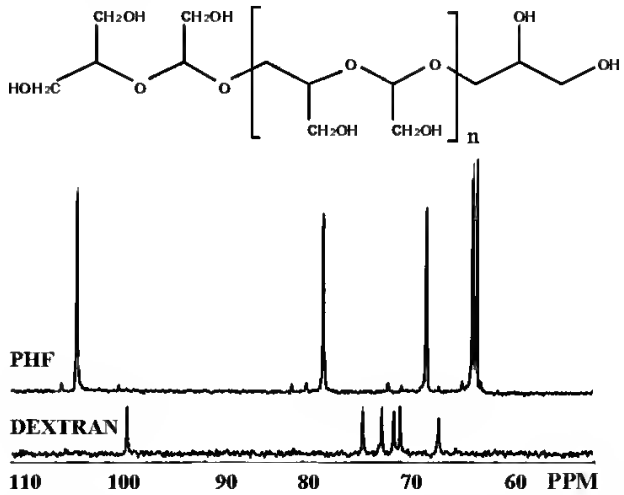


*Figure 3. Poly[hydroxymethylethylene hydroxymethylformal] (PHF), structure and $^{13}C$ NMR spectrum ; 293 K°, 10% solution, 9.4 Tl Brucker system, 100.619 MHz by $^{13}C$; proton decoupling, 45° flip angle, recycle delay 1.8 s (Dextran B512 spectrum is given as a reference).*

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The $^{13}C$ NMR spectrum of the final product (Figure 3) confirms the expected structure and shows that, unlike some other dextrans, where complete oxidation is blocked (presumably, as a result of formation of intramolecular hemiacetals), Dextran B512 can be completely oxidized with no identifiable residual cyclic structures. The phenol-sulfate analysis (xxi) also showed only traces (<<0.1%) of the residual carbohydrate.

One of our practical objectives was to develop a technique for large scale polysaccharide processing without significant depolymerization. The major concerns related to (a) possible inclusions of non-1->6 linkages in the poly-(1->6)-α-D-glucose main chain of Dextran B 512, that could be cleaved by periodate oxidation, and (b) relative instability of periodate-oxidized polysaccharides in alkaline media, which could result in depolymerization at the reduction stage (xxii). Preliminary tests showed that the commonly used versions of the periodate technique (developed for carbohydrate analysis and bioconjugate chemistry) afforded only small amounts of high molecular weight materials. Optimization of both the oxidation and reduction stages for minimal depolymerization resulted in consistently reproducible high yields of polymers with molecular weight distributions similar to the source dextrans (as determined by SEC HPLC) (xxiii,xxiv). Using flow dialysis as a prototype large scale technique for polymer purification and isolation, we obtained PHF with nearly theoretical yields for high molecular weight dextrans (MW>100 kDa). Low molecular weight polymers (MW=20-50 kDa) showed lower yields. The latter were attributed to inadequate polymer retention by low molecular weight cutoff filters, mainly at the final stage of PHF purification (PCF is reversibly associated in aqueous media, especially at 5<pH<7, which facilitates polymer retention by flow dialysis filters). Low molecular weight preparations of PHF were obtained with high yields via alternative procedures: (a) polymer purification by size exclusion chromatography, and (b) partial hydrolysis of 150-200 kDa polymers.

## Properties

Both polymers, the intermediate PCF (Figure 2, III) and PHF (Figure 3), were obtained in >99% pure form (by SEC HPLC) as colorless solid compounds.

PCF was found to be stable in aqueous media below pH≈9. Depending on the pH, PCF undergoes transitions that appear to be similar to the previously described for partially oxidized dextrans (xxv). At pH=4÷5, most aldehyde groups seem to exist in a gem-diol form. At lower pH the aldehyde absorption peak (267 nm) becomes apparent, and above pH 5 both enol and enolate forms are present (240 and 290 nm). Formation of the enol form appeared to correlate with significant intermolecular association at pH=5÷7. PCF was found to be soluble in water, dimethylsulfoxide (DMSO), dimethylformamide (DMFA), pyridine and water-alcohol mixtures, and insoluble in acetone, acetonitrile, dioxane, methanol, ethanol, glycerol, methylenechloride, toluene and triethylamine. Solubilization of dehydrated (lyophilized) preparations in water was slow, except at pH>7.

The reduced (polyalcohol) form, PHF, was found to be highly hygroscopic. Samples exposed to humid air were viscoelastic at ambient temperature. The apparent

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melting range of lyophilized PHF (MW=50-200 kDa) was within 100±20°C, depending on the molecular weight, and dramatically decreased after exposure to the ambient (humid) air. High molecular weight PHF is readily soluble in water, DMSO, DMFA and pyridine; slowly soluble in glacial acetic acid and ethyleneglycol, and insoluble in acetone, acetonitrile, dioxane, methanol, ethanol, glycerol, methylenechloride, toluene and triethylamine. Preparations with MW<5 kDa were soluble in methanol.

As expected, the stability of the PHF main chain was pH-dependent. While incubation at the neutral and high pH over several days did not change SEC elution profile, incubation at pH<7 showed significant fragmentation (Figure 4). In the presence of 50 mM sodium phosphate buffer, the hydrolysis rate at pH=3 was almost twice higher. Solubilization of crosslinked PHF gels in aqueous media showed an analogous pattern. At pH=7.5, both soluble and crosslinked PHF were resistant to a one hour incubation at 100°C.

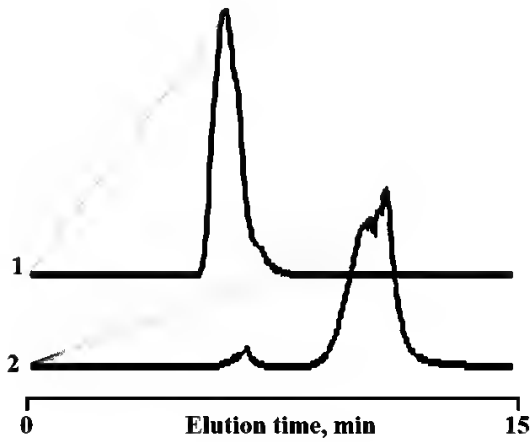


*Figure 4. Size exclusion HPLC profile of 200 kDa PHF before (1) and after (2) 4 days incubation at pH=3, 37°C.*

This pH dependence of main chain stability is valuable in several biomedical applications, where polymer-based products should be stable and functional in biological milieu (pH=7÷7.5) but undergo depolymerization after internalization by cells. Degradation of the cell-internalized polymer is important to avoid adverse effects associated with long-term polymer deposition in cells, in the first place in the glomerular mesangium and reticuloendothelial system (xxvi,xxvii).

Acidic conditions (pH≈5) are characteristic for the intracellular lysosomal compartment where polymers are transferred after internalization by cells. Therefore, cellular uptake of PHF-based preparations can be expected to result in non-enzymatic main chain hydrolysis at a moderate rate. This appears to be a significant advantage, as compared to several synthetic polymers, e.g., polyethyleneglycol, polyacrylates and vinyl polymers, which are hydrolysis-resistant. The final products of the PHF

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hydrolysis, glycerol and glycol aldehyde, have low toxicity; both are metabolized via major metabolic pathways. This may be one of the underlying reasons for the observed extremely low toxicity of PHF (see below).

## Derivatives

Modification of either polymer did not present significant difficulties. Due to the availability of well-developed methods for alcohol and aldehyde group modification, the reaction conditions can be selected such as to ensure the integrity of the polyacetal main chain (e.g., at 4<pH<9 in aqueous media). Although neither polymer is soluble in most organic solvents, several desirable lipophilic derivatives, e.g., PHF conjugates with lipids, can be successfully synthesized in suitable solvent mixtures (e.g., pyridine-DMSO or pyridine-methanol).

To investigate the technological flexibility of PCF/PHF system and to characterize PHF-based preparations, several model linear and branched forms of derivatized PHF, model gels and bioconjugates were successfully synthesized and studied in vivo. The examples are given below.

*PHF derivatization*

Direct derivatization of PHF through primary alcohol groups. The alcohol groups of PHF can be acylated or alkylated in DMSO, DMFA or in water. Acylation with diethylenetriaminepentaacetic acid monocycloanhydride in DMSO was utilized to obtain PHF modified with diethylenetriaminepentaacetic acid (DTPA), a chelating group suitable for polymer labeling with metal ions such as $^{111}In$ (radioactive γ-emitter). Indium-111 labeled preparations were used in biokinetics and imaging studies. Alkylation with epybromohydrine in water was utilized to produce model epybromohydrine-crosslinked gels (that were used to investigate the resistance of PHF-based matrix to hydrolysis).

Derivatization through terminal 1,2-glycol group was used for producing terminus-activated PHF. The 1,2 glycol is formed at the former reducing end of the polysaccharide chain (whereas at the former non-reducing end a 1,3 glycol is present), see Figure 3. The 1,2-glycol is readily transformed into active aldehyde group via periodate oxidation. For example, a terminus-activated polymer with apparent molecular weight of 3.6±0.4 kDa per aldehyde group ($I_2$ titration) was produced and subsequently conjugated with lipids (in pyridine-methanol media) and proteins (in water) (xxviii).

Derivatization through non-terminal glycol groups. Non-terminal 1,2-glycol groups were introduced into PHF structure via modification of the polysaccharide oxidation technique. Oxidation of the original dextran was ca. 10% incomplete (all carbohydrate rings were open but 10% of the C3 were not eliminated), so the product of subsequent reduction (PHF) contained 1 glycol per 20 functional groups. The glycol groups were further oxidized with periodate resulting in PHF comprising active aldehyde groups along the main chain. The latter were conjugated with several model reagents via aldehyde condensation with amino-, hydrazido-, aminooxy- and other groups (see below).

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Partial fragmentation of the PHF backbone with simultaneous incorporation of new functional groups was used to produce PHF with activated terminal groups. Treatment with mercaptopropionic acid in DMFA (mercaptolysis) resulted in fragments containing terminal carboxyls. The fragmented polymers were fractionated by precipitation (DMSO/chloroform or DMFA/acetone) and further subfractionated by HPLC. The terminal carboxylic groups were activated in DMSO with N-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide. The resultant polymer containing terminal N-oxysuccinimide ester group was precipitated and washed with chloroform and lyophilized. Terminal N-oxysuccinimido-PHF was used to produce soluble terminal graft copolymers (comb copolymers) with polyamines, e.g., with poly-L-lysine via direct reaction in water (xxiv), and conjugates with lipids (distearoylphosphatidylethanolamine, DSPE) via condensation in DMSO/pyridine mixture. The DSPE-PHF conjugates were used for liposome stabilization (xxviii).

*PHF derivatives via modification of aldehyde groups of PCF*

Modification of aldehyde groups of PCF (or PHF comprising aldehyde groups generated via glycol oxidation as described above) presents a set of synthetic approaches for producing a vast variety of PHF derivatives in mild conditions. For example, aldehyde groups can be conjugated in aqueous media with amines via formation of enamines with subsequent cyanoborohydride or borohydride reduction; this approach is widely used in protein immobilization on polymers (xxix,xxx).

Whenever conjugation through amines is not desirable, e.g., the reagent to be coupled with PHF has a biologically functional aminogroup, a variety of aldehyde group reactions with hydrazides, hydrazines, O-substituted hydroxylamines and 2-mercaptoamines (e.g., N-terminal cysteine) can be utilized. These reactions can be carried out in conditions where enamines are not formed (for example, in aqueous media at pH=4÷6).

Selectivity of aldehyde-mediated reactions opens the way to fast synthesis of complex functional conjugates, for example graft copolymers carrying multiple labels on the backbone (xxxi) and several cell-specific ligand groups (of one or more types) on the side chains. Aldehyde-mediated reactions can also be used for assembling complex PHF-based functional matrices e.g., for tissue engineering. Examples of PHF derivatization via aldehyde reactions are given below.

Partial derivatization of PCF was used to produce linear functionalized PHF derivatives and random-point PHF graft copolymers.

Linear PHF conjugate carrying fluorescein, DTPA and formyl-Met-Leu-Phe-Lys (f-MLFK, a chemotactic peptide) was synthesized via PHF condensation with cystamine ($H_2N$-$C_2H_4$-SS-$C_2H_4$-$NH_2$) and f-MLFK, with subsequent cystamine reduction and modification of the formed mercaptogroups with fluorescein maleimide (fluorescent label) and DTPA (chelating group for $^{111}In$). This preparation was used as

*Figure 5. The structure of fMLFK-DTPA-PHF conjugate. The PHF backbone (ca. 1 kDa chain fragment shown) is modified by fMLFK (black) and DTPA (light gray) at random positions.*

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a model cooperative vector for targeting formylpeptide receptors of white blood cells (xxxvii,xxxviii).

Random-point graft copolymers of PHF and DTPA-modified poly-L-lysine (backbone) were prepared, using previously developed technique (xxxi), via DTPA-Polylysine condensation with an excess of PCF, with subsequent reduction and separation of the unbound PHF. A dextran-polylysine graft copolymer was prepared analogously as a control for animal studies. The hydrodynamic size of both products, as determined by photon correlation light scattering, was 16±4 nm. Graft content was 20-25 molecules per backbone. Both copolymers were labeled with Indium-111 for animal studies.

**In vivo studies**

Because the central practical objective of this study was to develop a polymer with minimized interactions in vivo, we studied biokinetics of PHF and various PHF derivatives and attempted to identify the dose level at which toxic effects of PHF would become noticeable. Biokinetics provide valuable data on polymer interactions in vivo because, for particles and large macromolecules circulating in blood, blood half life is a mathematically exact measure of the overall polymer interactions with the biological milieu (xiv). Biologically inert ("stealth") polymers are expected to have insignificant accumulation in RES and other tissues. Low rates of tissue binding and uptake by cells result in a long blood half-life, except relatively small molecules (generally, MW<50 kDa) which can be cleared from blood via renal filtration.

Acute toxicity in mice. PHF of the highest molecular weight available at the time of the experiment (approximately 0.5-1 MDa) was used to minimize renal excretion that would mask the potential toxic effects. Although the injected dose reached 2 g/kg, all animals survived. After 32 days, all animals were alive, and their weights did not significantly differ from the control group (24±3 g vs. 25±2 g). None of the animals showed any noticeable symptoms of toxicity, including anaphylactoid reactions (e.g., paw edema) that develop in rodents in response to administration of many polymers, including dextran B 512. Absence of adverse reactions indicated that PHF interactions with immunocompetent cells and recognition proteins were biologically insignificant, which is in agreement with the underlying hypothesis. Administration of large doses of PHF-based preparations in rats and rabbits also did not cause any signs of toxicity nor anaphylactoid reactions.

Circulation of PHF was studied in normal anesthetized rats. Radiolabeled preparations were administered via tail vein. The initial biokinetics were studied by dynamic γ-scintigraphy (xxxii). Blood half-life of the low molecular weight [$^{111}$In]DTPA-PHF (50 kDa fraction) in rat was found to be 45 min (clearance via renal filtration). The polymer was cleared by 24 post injection, with very little accumulation in tissues (<0.05% dose/g in any tissue). The highest label accumulation (0.16% dose/g) was found in kidneys. The high molecular weight [$^{111}$In]DTPA-PHF (500 kDa fraction) demonstrated significantly longer circulation (blood half-life ca. 26 hr.), with almost even distribution among tissues. Accumulation in RES was only

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twice as high as in other tissues, and thus was related, most likely, to a higher rate of spontaneous endocytosis in RES, rather than to PHF recognition by RES phagocytes.

Biokinetics of graft copolymers. Biokinetics of graft copolymers depend (at high graft densities) on the structure of the graft, whereas the effect of sterically hindered main chain is minimal. The graft copolymer model is sensitive to cooperative interactions because several graft chains can interact with a substrate (e.g., functional components of cell surface) simultaneously. For example, multiple chains of dextran B-512 in dextran/polylysine graft copolymers (and dextran-coated nanoparticles) are readily recognized by lymph nodes and spleen phagocytes, whereas single dextran molecules are not (xviii,xxxiii).

Biokinetics of graft copolymers were studied in normal outbred rats as described above. A series of graft copolymers of PHF with different graft densities showed the following results. Terminal (comb) copolymers with graft densities of two, seven, and ten PHF chains per backbone showed blood half-lives of 5.4±0.3, 7.2±1.2, and 9.8±1.5 hours, respectively. The long blood half-lives at higher graft densities, where copolymer molecule interactions are mediated essentially by the side chains, indicated low overall level of cooperative interactions of PHF in vivo.

In the subsequent comparative study, random-point graft copolymer of dextran showed blood half-life of ca. 1.5 hr. and a highly characteristic uptake in lymph nodes and spleen, with somewhat lower accumulation in liver and kidneys. Graft copolymer of PHF with analogous structure showed a much longer 25.3±2.5 hr. blood half-life, and a dramatically lower uptake in RES (Table 1).

Thus, the results of in vivo studies showed that neither linear nor highly branched PHF derivatives were efficiently recognized by RES, unlike the original Dextran B512. In studies with partially oxidized dextran (xxiii), loss of recognition correlated with elimination of the rigid stereospecific structures of the carbohydrate molecule.

*Table 1. Biodistribution of Dextran and PHF graft copolymers in rat (% dose/g tissue), 24 hr. after intravenous administration (1 mg/kg body weight). From (xv).*

| Tissue | Graft | |
|---|---|---|
| | Dextran B-512 | PHF |
| Blood | 0.3 | 3.7 |
| Lymph nodes, paraaortic | 58.9 | 0.9 |
| Lymph nodes, mesenteric | 81.8 | 0.8 |
| Spleen | 19.9 | 1.3 |
| Liver | 9.0 | 2.1 |
| Kidney | 2.7 | 3.7 |
| Muscle | 0.1 | 0.4 |
| Heart | 0.3 | 0.9 |
| Lung | 0.2 | 1.2 |

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Biokinetics of PHF modified with chemotactic peptide was studied to evaluate PHF as a biodegradable "stealth" backbone polymer for targeted macromolecular drugs.

The model chemotactic peptide, f-MLFK, binds formylpeptide receptors of white blood cells. As a result, administration of labeled f-MLFK preparations results in label accumulation in the areas of white blood cell invasions, such as acute inflammations (xxxiv). Peptide conjugation with macromolecules hypothetically can open the way to dramatic improvements in pharmacokinetics by means of (1) regulating the blood clearance via decreasing the rate of renal and, possibly, RES clearance and (2) increasing the agent-leukocyte association constant via cooperative binding effect of multiple peptide molecules exposed on the carrier. The cooperative character of agent-leukocyte interaction suggested an additional opportunity to explore a (3) hypothetical thermodynamic discriminatory effect that is expected to result in a more selective agent association with leukocytes and suppression of non-specific interactions with other tissues. The improvements in biokinetics, however, would be diminished if the backbone polymer interactions prevailed in the overall conjugate interactions in vivo.

Biokinetics of [$^{111}$In]DTPA-mercaptoethylamino-PHF-fMLFK, 15 and 70 kDa (Figure 5), was studied in a rabbits. Animals were normal or bearing focal bacterial inflammation induced by inoculation of E.Coli (clinical isolate) in thigh muscle. $^{111}$In-labeled PHF-DTPA and monomeric DTPA-fMLFK were used as control preparations. Images were acquired over a 20 hr. period, followed by a biodistribution study.

The blood clearance rate of the 15 kDa preparation was fast; approximately 80% of activity was cleared from blood during the first 15 minutes through kidneys; the rest was cleared with a half-life of 45 min. The 70 kDa preparation showed half-life of 2 hr. with no initial fast phase. Both preparations significantly accumulated in the infection site. Scintigraphic images of the final biodistributions are shown in Figure 6.

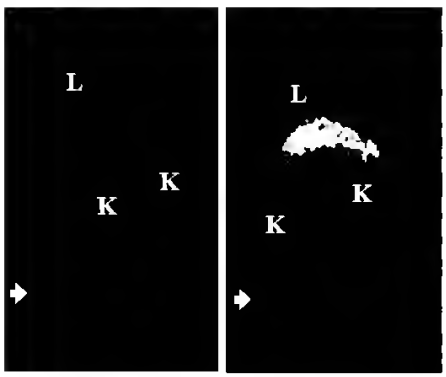


*Figure 6. Whole body scintigraphic images of rabbit (inflammation model).*

*Anterior view, 20 hr. after administration of radiolabeled f-MLFK (left, control) and f-MLFK-PHF conjugate (right). K: kidneys; L: liver.*

*Note accumulation of both preparations in the inflammation (arrow), and significantly lower out-of-target accumulation of the f-MLFK-PHF conjugate, especially in kidneys.*

The biodistribution data showed that immobilization of multiple f-MLFK molecules on PHF did not increase label accumulation in RES as compared to monomolecular f-

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MLFK, and decreased accumulation in kidneys by 80% (xxxvii). This study showed feasibility of PHF (from both technological and biological points of view) as a backbone polymer in targeted bioconjugates.

## Discussion

The goal of this study was to determine whether a polymer emulating common acyclic structures of bilogical interface carbohydrates (hydrophilic polyacetal) would have a combination of properties close to an "idealized" biomedical material, such as: "inertness" in vivo, biodegradability of the main chain, low toxicity, and technological flexibility.

The model hydrophilic polyacetal, PHF, was produced via complete elimination of carbon 3 from carbohydrate residues of poly-(1->6)- α-D-glucose main chain of Dextran B 512. The blood clearance rates of PHF and PHF-protected macromolecules (graft copolymers) were close to that of similarly structured derivatives of polyethyleneglycol, (xxxv) which is currently the "gold standard" of biological inertness, and significantly longer than of analogously structured derivatives of dextran B-512 (xxxvi).

The potential advantages of hydrophilic polyacetals, as compared with polyethyleneglycol, are biodegradability and availability of readily modifiable groups along the main chain, which opens the way to producing various functional conjugates (xxxvii,xxxviii).

Advantages of polyacetals as compared to polysaccharides relate to both biological functionality and safety. For example, Dextran B512, (a.k.a. "pharmaceutical dextran"), which is known as one of the least biologically active polysaccharides (xxxix), is a product of a microorganism (*Leuconostoc Mesenteroides*). Dextran is known to produce anaphylactoid reactions that are mediated by immunoglobulins specific to isomalto-oligosaccharides (xl). The origin of the immunity is unknown; however, it has been shown recently that *Streptococcus Sanguinus*, an oral streptococcus prevalent in dental plaques (xli), produces isomalto-oligosaccharide containing lipoteichoic acid (xlii,xliii). The latter was shown to bind recognition proteins of plasma (xliv) and stimulate immunocompetent cells (xlv,xlvi). Therefore, *S. Sanguinus* can potentially induce production of oligoisomaltose-reactive antibodies, and the associated sensitivity to dextran-containing preparations, practically in any individual. Obviously, biomaterials lacking receptor-recognizable domains and antigenic determinants of wide-spread bacterial species would convey a much lower risk of anaphylactoid reactions.

## Conclusion

The experimentally determined properties of the synthesized model acyclic hydrophilic polyacetal (PHF) were in a good agreement with the hypothesis that polymers obtained via partial emulation of polysaccharides may have an excellent

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combination of useful features. Properties of PHF suggest the potential utility of polymers of this type in pharmacology and bioengineering, for example as structural or protective components in macromolecular drugs, drug delivery systems, and templates for tissue engineering. Development of carbohydrate-derived and fully synthetic hydrophilic polyacetals may become a promising direction in the development of new biomedical materials.

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